Behavioural Brain Research 261 (2014) 134–139

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Impaired imprinting and social behaviors in chicks exposed to mifepristone, a glucocorticoid receptor antagonist, during the final week of embryogenesis

Hideo Nishigori[a,*], Keisuke Kagami[a], Hidekazu Nishigori[b]

[a] Department of Pharmacotherapeutics, School of Pharmacy, Iwate Medical University, 2-1-1 Nishitokuta, Yahaba, Shiwa-gun, Iwate 028-3694, Japan
[b] Department of Obstetrics, Tohoku University Hospital, Sendai, Miyagi 980-8575, Japan

HIGHLIGHTS

- Chicks treated with mifepristone during embryogenesis impaired social behaviors.
- Mifepristone treatment during embryogenesis impaired social behaviors of hatchlings.
- Glucocorticoid receptor dysfunction during embryogenesis impaired social behaviors.
- Glucocorticoid receptor dysfunction during embryogenesis altered brain development.

ARTICLE INFO

Article history:
Received 17 September 2013
Received in revised form
21 November 2013
Accepted 24 November 2013
Available online 22 December 2013

Keywords:
Glucocorticoid
Mifepristone
Filial imprinting
Prenatal exposure
Chick embryo
Social behavior

ABSTRACT

The effects of glucocorticoid receptor dysfunction during embryogenesis on the imprinting abilities and social behaviors of hatchlings were examined using "fertile hen's egg-embryo-chick" system.
Methods and results: Of embryos treated with mifepristone (0.4 μmol/egg) on day 14, over 75% hatched a day later than the controls (day 22) without external anomalies. The mifepristone-treated hatchlings were assayed for imprinting ability on post-hatching day 2 and for social behaviors on day 3. The findings were as follows: imprinting ability (expressed as preference score) was significantly lower in mifepristone-treated hatchlings than in controls (0.65 ± 0.06 vs. 0.92 ± 0.02, $P < 0.005$). Aggregation tests to evaluate the speed (seconds) required for four chicks, individually isolated with cardboard dividers in a box, to form a group after removal of the barriers showed that aggregation was significantly slower in mifepristone-treated hatchlings than in controls (8.7 ± 1.1 vs. 2.6 ± 0.3, $P < 0.001$). In belongingness tests to evaluate the speed (seconds) for a chick isolated at a corner to join a group of three chicks placed at the opposite corner, mifepristone-treated hatchlings took significantly longer than controls (4.5 ± 0.4/40 cm vs. 2.4 ± 0.08/40 cm, $P < 0.001$). In vocalization tests, using a decibel meter to measure average decibel level/30 s (chick vocalization), mifepristone-treated hatchlings had significantly weaker vocalizations than controls (14.2 ± 1.9/30 s vs. 26.4 ± 1.3/30 s $P < 0.001$). In conclusion, glucocorticoid receptor dysfunction during the last week embryogenesis altered the programming of brain development, resulting in impaired behavioral activities in late life.

1. Introduction

Environmental toxicants and various stresses can influence the intrauterine hormonal environment and endocrine system of a fetus, affecting programming during embryogenesis and leading to presence of disorders soon after birth or later in life [1]. We have been interested in examining the roles of thyroid hormones (THs) and glucocorticoids (GCs) during embryogenesis in avian leading to imprinting abilities and social behaviors after hatching. Previously, to evaluate the harmful consequences of hypothyroidism during embryogenesis on hatchling's behaviors, we used the "fertile hen's egg-embryo-chick" system as an animal model [2,3]. The reasons are as follows: (1) Hatchlings have imprinting abilities that can be used to evaluate the neural basis of memory formation [4,5], and they move with their legs and vocalize, so their social behaviors can be easily determined by SSST (the chick's social-separation-stress test). The SSST was designed to evaluate the chick's willingness to form groups from isolated corners (evaluating belongingness and aggregation) and communicate with each other by vocalization [6,7]. (2) Chick embryos can be treated with drugs such as methimazole, an inhibitor of thyroid hormone synthesis, to directly produce the hormonal insufficiencies during embryogenesis,

* Corresponding author. Tel.: +81 19 651 5111; fax: +81 19 698 1852.
E-mail address: hnishigo@ar.wakwak.com (H. Nishigori).

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http://dx.doi.org/10.1016/j.bbr.2013.11.039

without maternal influences [2,7]. (3) The time-frame of brain development in chicken is similar to that in human, and brains of both chicks and human newborns are well developed at hatching and birth, respectively [8–11], but different from brain development in rodents, which is essentially completed between postnatal months 1 and 2 [11,12].

We have recently demonstrated that chick embryos treated with methimazole on day 14 hatched 3 days later than controls, and the hatchlings showed significantly lower scores in imprinting ability [2] and social behavioral activities than controls [7]. We therefore proposed that combining the fertile hen's egg-embryo-chick system and SSST animal models would be useful models for evaluating the unfavorable influences of prenatal stress on postnatal and later-life behaviors independent of maternal factors.

GCs and THs are also thought to play crucial physiological roles in prenatal programming and are necessary for the normal differentiation and maturation of various tissues, including the brain and other components of the nervous system. Several animal studies have suggested that high levels of GCs due to stressful events or exogenous administration during pregnancy caused reduced birth weight and induced hypertension, hyperglycemia, and behavioral abnormalities in the offspring [13–17]. These observations suggested that excessive GCs during embryogenesis, which is caused by stressful events, may cause unfavorable effects on the programming of fetal brain development, possibly leading to emotional and behavioral problems in children and later in life [18]. However, there is still little information regarding the influence of GC dysfunctions on neural development during embryogenesis and their effects on learning, memory and social behaviors after birth.

In chick embryos, the hypothalamo-pituitary-adrenal axis and neuroendocrine feedback control of serum GCs are established before the last week of development [19], and GC receptors (GRs) in the brain become functional by this time point [20–22]. To explore the effects of GC deficiency during the last week of development while maturation of the neural system proceeded [6–8], we used mifepristone, a specific GC antagonist in avian species [23]. Treatment with mifepristone can prevent GC-induced elevation of glucose and lipids in the blood and cataract formation in chick embryos [24,25]. Additionally, we found that chick embryos treated with mifepristone on day 14 hatched a day later than the controls [25,26] but without abnormalities in external appearance.

In the present paper, we examined the behaviors of mifepristone-treated hatchlings and found that they scored significantly lower on tests of imprinting abilities and social behavioral activities than controls, and we discuss the importance for normal neural development of both GCs and THs during the final week of chick embryogenesis.

2. Materials and methods

2.1. Preparation of fertilized eggs and administration of mifepristone

The procedures were carried out as described previously [6,7,26]. Briefly, fresh fertilized white Leghorn eggs (purchased from Koiwai Farm. Ltd., Japan) were incubated in an incubator (Model P05: Showa Incubator Company, Saitama, Japan) at 37.5 °C and 68% relative humidity, with rocking through a range of 30° in a 30-min cycle. The first day of incubation was termed "day 0". The eggs containing live embryos, selected by a pen light, were drilled through the shell in an air chamber with a gimlet and tweezers. Then, on day 14, they were treated with mifepristone (Sigma, 0.4 μmol suspended in 200 μL 2% Tween 20/egg). Correspondent control eggs received equivalent amounts of vehicle. After drug administration, the hole was covered with Scotch tape, and the eggs were returned to the same incubator.

The care of the animals and their sacrifice using lethal doses of carbon dioxide gas were performed according to guidelines set forth by the Animal Care and Use Committee of the Iwate Medical University and the National Laws on the Protection of Animals.

2.2. Imprinting behavior testing

On the day before hatching (embryonic day 20 or 21 for the vehicle- and mifepristone-treated groups, respectively), the eggs were transferred to individual plastic boxes in which they were physically and visually isolated from each other, and they were then transferred to another incubator for hatching. Imprinting behavior was evaluated by our previous method [2], based on the procedure described by Maekawa et al. [5]. Briefly, 1-day-old chicks (P1; postnatal day 1), hatched individually in dark compartments, were transferred to the apparatus for the imprinting test, and exposed to a red rotating training object for 150 min in the presence of light. The following day, the trained chicks, housed in dark compartments, were exposed to the training object and a novel blue object in the following counterbalanced sequence: training/novel/novel/training. Each session lasted for 5 min, and sessions were separated by approximately 10 s of darkness. The number of revolutions of the running wheel toward and away from the training or novel object was recorded each time, and the preference score (PS) for the training object was calculated as follows: running toward the training object/(running toward the training object + running toward the novel object).

2.3. Apparatus for social behavior testing

Apparatuses (including photographs) and procedures for behavior testings were detailed in our previous paper [6]. Apparatus A (open field): a cardboard box of 45 cm × 45 cm × 24 cm ($l \times w \times h$) with a paper towel on the floor. Apparatus B, for aggregation behavior test: apparatus A plus 4 cardboard fences placed 18 cm away from each corner to create triangular spaces (18 cm × 18 cm × 25 cm). Apparatus C for belonging behavior test and vocalization test: apparatus A with two triangular corners, in which one triangular space was made with a cardboard fence as in apparatus B and the opposite triangular space, was made with a stainless mesh placed 18 cm away from the corner.

2.4. Procedures for behavioral testing (SSST)

On day 18 for controls or day 19 for the mifepristone-treated group, five or six eggs were transferred from the rolling rack to a stationary plastic box in the incubator. A group of chicks was hatched and housed together in a plastic box in the incubator and exposed to the room light for 10 min on P1 and P2. The tests were performed on P3. All experiments were performed at room temperature (25–26 °C).

2.4.1. Surface righting test

A chick was placed on its back and gently held by its wings for 1 s before being released. The time taken by the chick to right itself was recorded using a stop-watch.

2.4.2. Aggregation behavior test

Of the five or six chicks hatched in the plastic box, four were randomly selected, released into the open field as a group for 1 min, and transferred individually to the isolated corners in apparatus B. After the cardboard sections were simultaneously removed, aggregation behavior was recorded with a video camera. Time (seconds) to form groups of two, three or four chicks was recorded using a